

# Extended Summary

## SCI Pesticides Group Symposium

### Interference with the Structure and Function of Biological Membranes

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#### Hormone Receptors at Plant Cell Membranes

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The biological action of any plant hormone is thought to be initiated by its binding to a specific recognition site, or receptor. In animal cells, both soluble and membrane-bound classes of receptor are well-characterised. In plants, no soluble hormone receptor has yet been unambiguously identified, although a zeatin-binding protein with many characteristics appropriate to a cytokinin receptor has recently been reported.<sup>1</sup> For all other native plant hormones, as well as for the growth regulators fusicoccin and phytotropins, there is evidence of receptors associated with the plasma membrane or other cellular membranes, though in some cases this evidence remains largely presumptive.

With both gibberellins and abscisic acid (ABA) there are data and theoretical arguments to implicate intracellular as well as extracellular sites of perception.<sup>2</sup> At the biochemical level, photoaffinity receptor labelling approaches have been applied with each of these hormones,<sup>3,4</sup> though the abscisic acid work, described in a single 1984 paper, still awaits confirmation.<sup>4</sup> The simplest plant hormone is ethylene, and biochemical approaches to receptor isolation have been pursued in the laboratories of Sisler in the USA<sup>5</sup> and Hall in the UK.<sup>6</sup> During the last few years, molecular genetic analysis of *Arabidopsis* ethylene response mutants has

implicated a kinase cascade in ethylene signalling.<sup>7</sup> It is not yet clear whether the two approaches will converge, but definitive identification of an ethylene receptor can be expected in the near future.

Phytotropins are synthetic inhibitors of auxin transport having certain molecular features in common.<sup>8</sup> Binding sites for these molecules, first reported in 1971,<sup>9</sup> are found in the plasma membrane, possibly associated with the cytoskeleton,<sup>10</sup> and are thought to function in connection with the auxin efflux carrier. The sites have not yet been purified, although a 23 kDa polypeptide has been identified in plasma membrane fractions by photoaffinity labelling.<sup>11</sup> Another growth regulator normally foreign to plant cells is the fungal toxin, fusicoccin, which evokes a number of auxin-like responses, including H<sup>+</sup>-ATPase activation. Again, fusicoccin receptors are found in the plasma membrane of diverse species and have been studied for many years. Remarkably, three laboratories reported independently in 1994 that purified fusicoccin receptor preparations contain 30 kDa proteins belonging to the 14-3-3 protein family.<sup>12–14</sup> Such proteins are widely distributed in eukaryotes and have been implicated in a variety of signalling functions. There is some debate as to whether primary fusicoccin perception is a function of the 30 kDa protein (actually a protein doublet) or of a 90 kDa species, found in maize preparations.<sup>15</sup> However, this is clearly an exciting time for fusicoccin signalling and further rapid advances can be expected.

Auxin-binding proteins (ABPs) have been the most widely studied and one such protein, ABP1 of maize, is now generally regarded as a *bona fide* auxin receptor.<sup>16,17</sup> First studied in the membrane-associated state,

it has been purified, cloned, sequenced and subjected to extensive antibody analysis. The protein has a C-terminal endoplasmic reticulum (ER) retention sequence, consistent with its predominant cellular location. The C-terminal region has also been implicated in signal transduction,<sup>18</sup> while auxin agonist antibodies have identified an important part of the auxin-binding domain.<sup>19</sup> Despite the predominant ER location, electrophysiological analysis points to the presence of functional ABP1 at the exterior face of the plasma membrane and such a population has recently been imaged and found to undergo auxin-induced clustering.<sup>20</sup> The mechanism by which a small fraction of this ER-targeted protein reaches the cell surface is still unclear. ABP1 has been expressed in the insect-baculovirus system in a form that is correctly targeted, folded and glycosylated,<sup>21</sup> opening the way to crystallisation. An important recent development in auxin signalling has been the cloning of the *AUX1* gene of *Arabidopsis*. This appears to encode a protein with at least seven transmembrane spanning domains<sup>22</sup> which is likely to be involved in auxin signal transduction. Whether this will prove to act independently of ABP1 or in concert is an intriguing question that awaits resolution.

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